Chapter

Repurposing Infectious Pathogen Vaccines in Cancer Immunotherapy

Matteo Conti

Abstract

Reports in the literature show that certain vaccines against infectious pathogens, can be effective in eliciting antitumor immune response when injected intratumorally. In mouse tumor models, intratumoral delivery of rotavirus, yellow fever, and influenza vaccines have been shown to also synergize with checkpoint inhibitors, in the leading immunotherapy in the clinical practice today. The combined approach can thus become a very promising novel strategy for anticancer immunotherapy. In humans, an attenuated poliomyelitis virus vaccine, a peptide-based vaccines against papilloma and one based on detoxified diphtheria protein have already been tested as intratumoral treatments readily. In those studies, the role of available anti-pathogen immunity appears an important element in mediating the activity of the repurposed vaccines against cancer. We therefore suggest how evaluating or eventually developing anti-pathogen immunity before intratumoral delivery could be helpful in repurposing infectious pathogen vaccines in cancer immunotherapy.

Keywords: cancer immunotherapy, cancer vaccines, repurposed vaccines, infectious agents vaccines, intratumoral delivery

1. Introduction

The immune system is physiologically able to detect and destroy abnormal cells and to curb the growth of clinically meaningful cancers [1]. However, during carcinogenesis, immune tolerance and immunosuppression mechanisms become more and more prevalent and critically detectable tumor masses start to appear in patients [2]. Recognized mechanisms are for instance: (1) genetic changes that make cancer cells less visible to the immune system [3], (2) release of specific molecular factors that subvert normal mesenchymal cells and certain immune cells into alleys [3, 4], (3) expression and/or overexpression of specific cancer cell surface proteins, such as checkpoint regulators, that directly inhibit immune cell activation [5].

Figure 1 provides an overview of the immunosuppressive interplay between a cancerous cell and the immune system into the tumor microenvironment. Cancer cells modulate their expression of receptors, release specific molecules and microvesicles in order not only to avoid destruction but to also recruit immune system components in their favor.

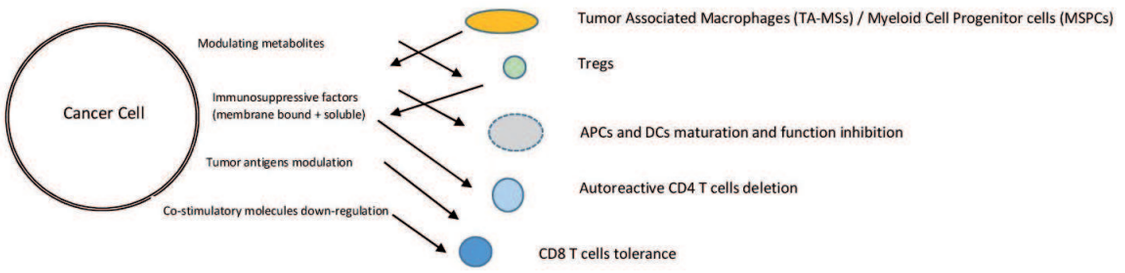

Figure 1.
Immune regulation within the tumor microenvironment.

Only quite recently scientists have started to learn how to interfere with such mechanisms and several types of immunotherapies (**Table 1**) have become available to clinicians [6, 7].

One main area of anticancer immunotherapy is that of adoptive cell transfer (ACT) therapy, which has shown remarkable activity against blood malignancies and even solid tumors [8–10]. In this therapy, immune cells are taken from patients' blood, selected, cultured, genetically modified and multiplied in the laboratory, before being reinfused to patients. Chimeric antigen receptor (CAR) T cells, in particular, are genetically modified in order to express specific very efficient receptors able to target cancer cells. These techniques actually require very special laboratories and expensive resources to be performed. Therefore, they are still out of reach for most of the patients worldwide.

Figure 2 is a schematic representation of chimeric antigen receptor (CAR) constructs delivered by retroviral transfection in T cell collected from patients and grown in culture. First-generation constructs employ a single-chain variable fragment (SCvf) connected by a linker to a transmembrane domain and an intracellular signaling domain. In second-generation constructs, one co-stimulatory domain (such as 4-1BB) has been added. In third-generation constructs, two co-stimulatory domains (such as 4-1BB or CD 134) have been employed. In fourth-generation constructs, a transgene protein for cytokines or chemokines has also been added. Despite this elaborated design, much research is still needed in order to improve CAR T cells efficacy and limit or control their toxicity.

Approved drug	Immunotherapeutic category
Nivolumab, pembrolizumab	Anti-PD-1 monoclonal antibodies
Atezolizumab, darvalumab, avelumab	Anti-PDL-1 monoclonal antibodies
Ipilimumab	Anti-CTLA-4 monoclonal antibodies
Sipuleucel-T	Dendritic cell-based vaccines
Tisagenlecleucel, axicabtagene ciloleucel (CD19 targeting)	CAR T cells
Talimogene laherparepvec	Oncolytic viruses
recombinant IL-2 and INFa	Immunostimulants
Imiquimod (TLR7 agonist)	Toll-like receptor agonists

Recently FDA-approved immunotherapies (left column) with indication of respective immunotherapeutic categories (right column).

Table 1.
Recent milestone drugs approved for immune oncology.

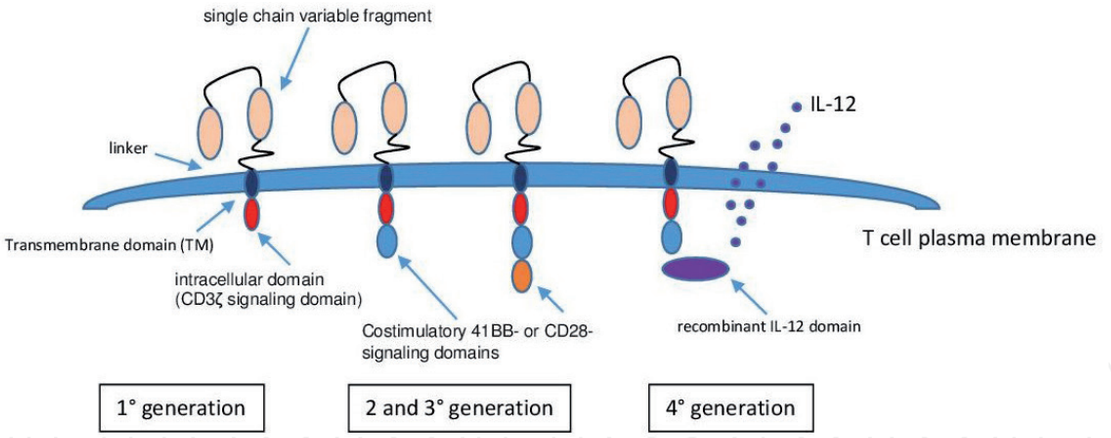

Figure 2.
Chimeric antigen receptor (CAR) constructs.

Checkpoint inhibitors (CIs) are monoclonal antibodies developed to specifically target checkpoint regulators that are responsible of immunosuppression by cancer cells in many cases. They are arguably becoming the most successful agents in the clinical practice. Some of them are already approved by regulatory agencies and broadly used in oncology practice (cf. **Table 1**). They show considerable efficacy, albeit still in a small percentage of patients, and much research is needed in order to improve their efficacy, avoid resistance development by cancer cells, and, also, reduce their systemic toxicity [10–12].

According to various reports, CIs efficacy is very much dependent on the presence and the number of tumor-infiltrating lymphocytes (TILs) in tumor lesions [5] and so various strategies in clinical trials try to increase tumor recognition by the immune system, to turn cold turn cold (immunosuppressed) tumors into hot (immunoactive) ones [11, 13].

CIs are combined with chemotherapy which, inducing cytolysis and release of neoantigens, can trigger an activation of the immune system. The problem with this approach is that most chemotherapies are myelotoxic and immunosuppressive in nature, to the point that the immune system can become so weakened and impaired to effectively fight against left-over cancer cells. Chemotherapeutic agents, such as cyclophosphamide and gemcitabine, having relatively lower myelotoxic effects, appear among the best candidates for this approach [14–16].

Radiotherapy is also employed because it is able to cause immunogenic cell death, cytolysis, and neoantigen release [14, 17–19]. In principle, it should induce lesser systemic immunosuppression than chemotherapy. In addition, the so-called abscopal effect enable extending immunotherapeutic effects to nonirradiated lesions [20].

Other physics-based techniques, such as cryotherapy, radiofrequency, electrochemotherapy, phototherapy, chemoembolization, and others, can synergize with CIs as well, by causing release of neoantigens secondary to induced cancer cell death [20–22].

Another interesting area of combination therapy with CIs is that with intratumoral delivery of pathogen-associated molecules, which could be used to activate the immune system inside the tumor microenvironment. This approach is the focus of the next sections of this writing. It must be pointed out that it heavily relies on the possibility of delivering molecules directly into tumor lesions by interventional radiology/oncology techniques, because if delivered systemically these molecules would be neutralised by the immune system before they could even reach their target [23–25].

2. Intratumoral delivery of pathogen-associated molecules

Probably the most famous historical account on the use of pathogens to treat tumors is that of William Coley. He was the first to report the observation that soft tissue sarcoma could naturally regress after bacterial infections. Facing cases in his clinical practice, he then proceeded to cause such risky infections on purpose, using bacterial-derived material (Coley's toxins) to locally inject tumor masses, observing successful tumor regression in some case [26–28].

Another well-known example of an infectious pathogen used for local tumor treatment is that of Calmette-Guerrin bacillus for transurethral instillation in urothelial carcinoma [29].

We are today able to deliver much better defined preparations of engineered recombinant viruses and bacteria into tumors, as well as of a variety of pathogen-derived molecules to trigger the immune response. In general, the presence of pathogens is sensed by specialized immune cell receptors [30].

Main families of these receptors are: toll-like receptors (TLRs) on the plasma membrane and in endosomal compartments, cytoplasmic receptors for viral nucleic acids, such as retinoic acid-induced gene 1 (RIG-I), melanoma differentiation-associated protein 5 (MDA-5), stimulator of interferon genes (STING), and the intracellular nucleotide-binding oligomerization domain-like receptors (NOD) family of receptors. They are also entangled and shared by those that detect stressful cell death (DAMPs) secondary to infectious conditions. Therefore, many types of PAMP and DAMP agonists are under study alone and/or in combination with other immune system activators, such as CIs but also immune cell direct activators and growth stimulators.

Pharmaceutical formulations of polyinosinic: polycytosinic acids (poly I:C) can mimic double-stranded RNA molecules of viral origin sensed by the endosomal TLR3 receptors and by the intracellular RIG-I and MDA-5 sensors, and have been studied in transplantable mouse tumors, yielding good results in combination with checkpoint inhibitors [31]. Stabilized poly I:C formulation (poly ICLC, Hiltonol) has been employed for intratumoral delivery as monotherapy and/or in combination, in a few clinical trials [31–33].

TLR7/8 natural agonists imiquimod and resiquimod have been used against basal cell carcinoma [34, 35], melanoma, and other skin neoplasms [36] as well as against common warts [37, 38]. Local imiquimod has also been used in combination with radiotherapy for breast cancer in the clinic [39]. Intratumoral administration of TLR7/8 agonist NKTR-262 is being studied in patients with locally advanced or metastatic solid tumors (NCT03435640). Preliminary results from the phase I/II REVEAL trial noted a disease control rate of about 50% [40].

Intratumoral delivery of TLR9 agonists CpG oligonucleotides has been employed very successfully in mouse models and seen to be able to even determine cancer eradication by the immune system [41]; but, it failed to provide clear benefits in clinical trials [42, 43]. A combination of a CpG oligonucleotide with an agonistic anti-OX40 antibody intratumorally administered both in syngeneic transplanted and genetically determined tumor models was able to induce complete tumor eradication in mice [44] and the combination of these two agents (namely SD-101 and BMS 986178) is now under testing in ongoing trial against a variety of tumors (NCT03831295).

Intratumoral injection of STING-agonist dinucleotides can be another way to unleash the curative tumor response against transplantable mouse models [45]. Human STING agonist adu-s100, for instance, is undergoing clinical development (NCT 02675439).

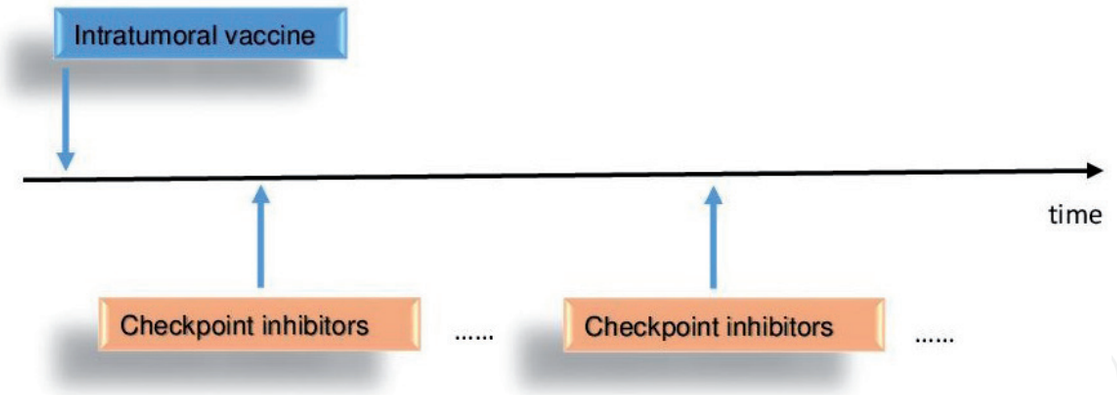

Figure 3.
Schematic diagram of the neoadjuvant intratumoral delivery of a therapeutic vaccine.

All these immune activators should be delivered intratumorally, ideally in a neoadjuvant setting, in order to synergize with current systemic immunotherapies (**Figure 3**).

3. Intratumoral delivery of pathogens

Entire pathogens, in particular recombinant oncolytic viruses, have been engineered to sustain selective replication into malignant cells [46, 47]. However, experience with the use of these oncolytic viruses, originally thought as cytolytic agents, has shown that antitumor immune response against viral-infected cells is a fundamental factor for their anticancer efficacy [48]. Therefore, modern viral vectors are genetically engineered to also express cytokines and other immune stimulating factors [49].

Vaccinia and herpes viruses have proven most effective when engineered to encode for immune-promoting genes such as interleukin 12 (IL-12) and granulocyte macrophage colony-stimulating factor (GM-CSF) [49, 50]. These agents are dramatically enhanced in their therapeutic performances by concomitant administration of PD-1/PDL-1 and CTLA-4 blocking Abs [51] as well as anti-CD137 or anti-OX40 agonist Abs [52–54]. Vectors based on vaccinia virus encoding GM-CSF (JX-594) are also under clinical development with promising results [55, 56].

The most successful agent so far in this category is herpes virus (HSV-1) modified to encode GM-CSF, named T-vec (talimogene). It has been granted Food and Drug Administration approval for unresectable melanoma [57]. Essentially, engineered pathogen preparations are delivered intratumorally in the neoadjuvant setting (essentially according to the scheme in **Figure 3**).

4. Clinical trials on intratumorally delivered pathogens and pathogen-associated molecules

Immunotherapies do not come without adverse effects and complications. In addition, patients have their own peculiarities and it is vital that clinicians identify the best therapeutic options for each one of them. In this light, there are various ongoing clinical trials evaluating intratumoral immunotherapies based on pathogen-associated molecules, alone or in combination with other therapies [25]. Poly-ICLC (Hiltonol) is in phase I against prostate cancer (NCT03262103); TLR7 agonist (Imiquimod) is in phase III against melanoma (NCT01720407);

TLR9 agonist (CMP-001) in combination with Anti-PD-1 (Nivolumab) is in phase II against melanoma and lymph node cancer (NCT03618641); and TLR8 agonist (VTX-2337) in combination with Anti-PD-1 (Tislelizumab) is in phase I against head and neck cancer (NCT03906526). JX-594 (Oncolytic virus) is in phase II against colorectal carcinoma (NCT01329809); and T-VEC (Oncolytic virus) is in phase II against melanoma (NCT02211131), in combination with Anti-PD-L1 (Atezolizumab) in phase I against breast cancer (NCT03802604), in combination with chemotherapy in phase I/II against breast cancer (NCT02779855), in combination with Anti-PD-1 (Pembrolizumab) in phase II against melanoma (NCT03842943), in combination with BRAF Inhibitor and MEK Inhibitor in phase II against melanoma (NCT03972046), in combination with radiotherapy in phase I/II against soft tissue sarcoma (NCT02453191), in combination with chemotherapy, radiotherapy, in phase I against rectal cancer (NCT03300544). Rilimogene galvacirepvec (PROSTVAC) in combination with Anti-PD-L1 (Atezolizumab) is in phase II against prostate adenocarcinoma (NCT04020094); GMCI (Adenovirus) in combination with radiotherapy, chemotherapy, is in phase II against pancreatic adenocarcinoma (NCT02446093); and HF10 (Oncolytic virus) in combination with Anti-PD-1 (Nivolumab) is in phase II against melanoma (NCT03259425). OrienX010 (Oncolytic virus) in combination with Anti-PD-1 (Treprizumab) is in phase I against melanoma (NCT04197882).

5. Intratumoral delivery of repurposed vaccines

Success with T-vec and other immune-boosting viruses have prompted various groups to search among routinely available attenuated viral vaccines to find other therapeutic options. The advantage of repurposing such approved and marketed agents is that clinical development would be much simplified, based on well-established safety records [58].

Commercially available attenuated rotavirus vaccines are preparations of double-stranded RNA attenuated strains. They are very potent stimulators of the nuclear factor kappa-light-chain-enhancer of activated B cells and type I interferon pathways. Interestingly, this stimulation is independent from the innate Toll-like immune receptors but dependent on RIG-I, which is able to detect intracytoplasmic dsRNA. Furthermore, rotavirus exerts cytocidal effects on adult and pediatric cancer cell lines in culture with features of immunogenic cell death. Intratumoral delivery to mouse bearing transplantable tumors, including pediatric syngeneic neuroblastoma models, elicited clear therapeutic effects mediated by natural killer (NK) cells and CD4 and CD8 T cells. In models of tumors refractory to checkpoint inhibitors, intratumoral rotavirus enabled to overcome resistance. Prevaccination of mice prior such intratumoral virotherapy did not spoil its efficacy [59].

A vaccine based on the 17D strain of the yellow fever virus, commonly used for travelers and dwellers in endemic areas, was demonstrated cytocidal for a large panel of human and mouse tumor cell lines. Its intratumoral administration was able to delay tumor progression by activating CD8 T cell-mediated immunity and some measurable effect could be observed against non-injected tumor lesions [60]. Additive effects with systemic immunostimulatory monoclonal antibodies directed to anti-PD1 or anti-CD137 were demonstrated. Very importantly, efficacy was potentiated by previous vaccination against the same virus in a manner dependent on T-cell antiviral acquired immunity [61].

Intratumoral injections of anti-influenza vaccines were also demonstrated to elicit immune-mediated antitumor activity in melanoma, in a series of experiments with

syngeneic transplantable tumor model [62]. Most surprisingly, only unadjuvanted inactivated influenza vaccines were able to generate such antitumor efficacy. Indeed, squalene-based adjuvanted influenza vaccines were losing their antitumor activity because adjuvants were recruiting interleukin-10-secreting B regulatory cells [62]. The detrimental role of adjuvants was observed in another seminal study when analyzing the cause of a lack of therapeutic enhancement of anti–CTLA-4 monotherapy by concurrent vaccination with gp100 peptide in incomplete Freund's adjuvant (IFA) [63].

Genetically engineered poliovirus vaccine antitumor activity was studied in mice a few years ago [64]. It has later been moved to a phase I clinical trial for recurrent glioblastoma with interesting results [65]. In this study, patients were pre-immunized with the vaccine against poliomyelitis and then treated intratumorally with the genetically engineered virus. The role of previously developed immunity was important for successful activation of immunity against tumors treated locally [65].

In an older phase I-II trial, a recombinant nontoxic diphtheria protein (CRM197), used in many common vaccines, was used to treat a variety of accessible tumors by local delivery. Response was observed in patients that had an already developed immunization (measured both by IgG titer and delayed type hypersensitivity) against diphtheria [66].

Since immunosuppression mechanisms are in place in the tumor microenvironment [67], from these examples it is clear that an effective immunity developed outside tumors could enable a better response when antigens are later delivered intratumorally. The fact that developing immunity outside the tumor microenvironment is a valuable strategy has been also demonstrated in the case of a new neoantigen vaccine formulation. In fact, the biomaterial-based vaccine prevented the engraftment of AML cells when administered as a prophylactic and when combined with chemotherapy, and eradicated, established AML even in the absence of a defined vaccine antigen [67, 68].

As a last example, a recent Report in JAMA Dermatology suggested that Gardasil®9 might be employed for cancer treatment. Cutaneous basaloid squamous cell carcinoma (BSCC) was eradicated by intratumoral administration of the vaccine. Preventive systemic immunization was performed by a standard initial dose and a booster one, followed by intratumoral delivery of the same vaccine into just a few of the largest lesions, injected monthly over the next months. During this relatively long period, even tumors that had not been injected went into complete regression. Notably, no recurrence was observed in the follow-up period (18 months). This report first presents clinical evidence that a prophylactic antiviral vaccine may be used as an effective immunotherapy for cancer [69].

All mentioned studies point out to the value of a therapeutic strategy outlined in **Figure 4**.

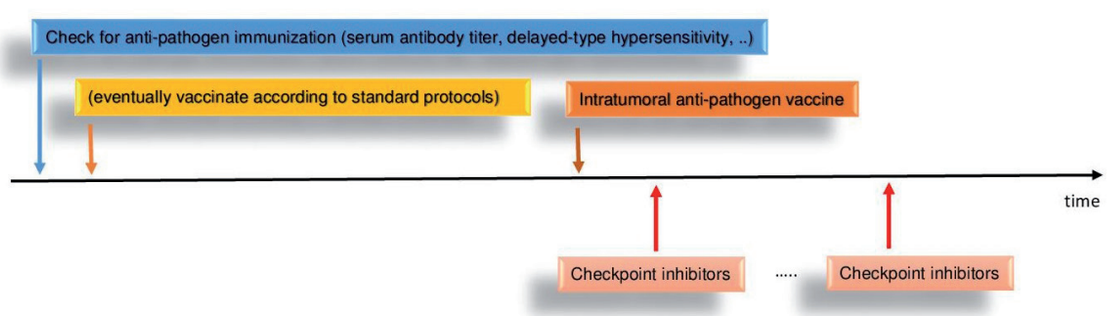

Figure 4.
Schematic diagram of the neoadjuvant intratumoral delivery of repurposed vaccines.

Available immunity against pathogen can be checked initially in patients by means of standard serological testing and/or delayed-type hypersensitivity testing. A standard vaccination protocol can be performed when required before starting intratumoral delivery of a corresponding vaccine. Afterward, timely standard delivery of other therapies (i.e., with systemic CIs) follows.

6. Future perspective

Developments in cancer immunotherapy during the last years have significantly increased our hopes for successfully treating different cancer types. However, the development of new, more effective anticancer immunotherapeutic agents and strategies urges a thorough understanding of the aspects that allow cancer cells to escape elimination by immune cells.

In addition, there are important clinical, industrial, regulatory, and economic issues that must be addressed, outside the realm of advances in cancer immunology and biology, and that would make all the difference between success or failure in real life. Under the clinical perspective, for instance, there is a strong need to develop a community of trained interventional radiologists/oncologists able to actually translate the presented approaches into practice. This is an issue basically in the hands of training centers and schools of medicine abroad. Of foremost relevance is also the involvement of the industry for all new approaches to actually become available to patients worldwide.

7. Conclusion

Designing of novel immunotherapies would require personalized approaches, tailored not only on patient's genetic profiles but also on immunologic tumor characterization. To overcome specific immune inactivation, vaccines against pathogens could become a usable tool in optimized combo-therapies, particularly with checkpoint inhibitors. The role of preexisting immunity on their efficacy has been observed in a few presented studies. In fact, immunization from previous vaccination or previous infections, developed outside the tumor microenvironment, can promote activity of intratumorally delivered preparations. In this light, we warrant future research on available and commercial vaccine preparations to be repurposed as anticancer therapeutic vaccines.

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Author details

Matteo Conti
Public Health Department, Ausl Imola, Imola, Italy

*Address all correspondence to: mttconti@gmail.com

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